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Diversity, distribution, biology, and predators of adelgids (Hemiptera: Adelgidae) in the conifer forests of Bhutan

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Forests provide critical ecological and economic services in Bhutan. After reports of a heavy adelgid infestation damaging trees in a forest nursery in Changaphu in 2019, a country-wide survey was conducted between 2020 and 2022. Eight adelgid species were found. Four were described elsewhere before this survey: *Adelges* (*Annandina*) *tsugae* Annand, *Ad.* (*Gilletteela*) *glandulae*, *Pineus* (*Pineus*) *armandicola* Zhang, Zhong and Zhang, and *P.* (*P.*) *wallichianae* Yaseen and Ghani. The remaining 4 were new species described in a separate paper: *Ad.* (*Cholodkovskya*) *changaphuensis* Havill and Brunet, *Ad.* (*Cassiadelges*) *coccipus* Havill and Brunet, *Ad.* (*Dreyfusia*) *densae* Havill and Brunet, and *Ad.* (*An.*) *lepsimon* Havill and Brunet. The distribution, DNA barcode diversity, and biology of each species are discussed here. In addition, fungi associated with *P.* (*P.*) *armandicola* from different sites were identified using internal transcribed spacer and 28S DNA sequences as phylogenetically diverged members of the sooty mold family Capnodiaceae, suggesting that the association may not be species-specific. Finally, the dipteran predators associated with adelgids were summarized as a potential source of biological control agents. Immature morphology and DNA barcodes identified 3 operational taxonomic units of *Lestodiplosis* (Cecidomyiidae), one each of *Episyrphus*, *Parasyrphus*, and *Melangyna* (Syrphidae), 1 *Leucopis* and 4 *Neoleucopis* (Chamaemyiidae). DNA barcodes also identified hymenopteran parasitoids inside some cecidomyiid specimens as Playtgastridae and inside some chamaemyiid specimens as Figitidae and Pteromalidae, highlighting the multitrophic nature of potential pest management.

Keywords: Eastern Himalayas, hemlock, fir, larch, pine, spruce

Introduction

Forests are the dominant ecosystem type in Bhutan, and they provide critical ecological and economic services. Forests make up an estimated 69.71% (2.68 million ha) of the total land area of Bhutan (Forest Monitoring and Information Division 2023). The country's dramatic elevation range, from 100 to over 7,700 m above sea level, drives its exceptional forest diversity. This altitudinal variation, coupled with differing rainfall patterns, results in 11 distinct agro-ecological zones and a wide array of forest types, including subtropical, broadleaf, coniferous, and alpine. Approximately 52% of Bhutan's land is protected, and about 5% is used for timber production (Forest Monitoring and Information Division 2023), leaving the vast majority of its forests largely undisturbed and rich in biodiversity. It is imperative to ensure that the mainstays of the country's economy that rely on forest resources such as hydropower and tourism are not impacted by disruption of ecosystem services by biotic and abiotic disturbances.

Adelgids (Hemiptera: Adelgidae) are a group of insects with approximately 70 described species worldwide (Favret et al. 2015, Chou et al. 2025, Havill et al. 2025), some of which are serious forest

pests. They have an ancient association with their conifer (Pinaceae) hosts (Havill et al. 2007) and exhibit a complex 2-yr holocycle with cyclical parthenogenesis, gall formation, and host alternation (Havill and Foottit 2007). Primary hosts, on which sexual reproduction and gall formation occur, are always in the genus *Picea*, and secondary hosts are in *Abies*, *Larix*, *Pinus*, *Pseudotsuga*, or *Tsuga*. Alates (sexuparae) that are produced on secondary hosts give rise to sexual forms that can only feed on *Picea* primary hosts. Therefore, the presence of sexuparae is strong evidence for a holocyclic life cycle when suitable *Picea* host species are available. Species or populations of adelgids that are anholocyclic exhibit entirely asexual reproduction and no host alternation (Havill and Foottit 2007, Favret et al. 2015). Anholocycles can occur either on primary or on secondary hosts. Anholocycles on *Picea* usually include a galling generation and alates, but there are exceptions with no gall formation and only wingless generations (Havill and Foottit 2007).

Although conifers constitute just 32% of forest area in Bhutan (Forest Monitoring and Information Division 2023), they are a major source of timber. In Bhutan, the potential host species in the genera that adelgids utilize are *Abies densa* Griff., *Larix griffithii* Hook. f., *Picea brachytyla* (Franch.) E. Pritz., *Picea spinulosa* (Griff.) A. Henry, *Pinus bhutanica* Grierson, D. G. Long and C. N. Page, *Pinus roxburghii* Sarg., *Pinus wallichiana* A. B. Jacks., and *Tsuga dumosa* (D. Don) Eichler (Grierson and Long 1983). *Pinus*, *Picea*, and *Tsuga* are the preferred conifer genera for timber harvesting in Bhutan (Forest Monitoring and Information Division 2023).

Bhutan has not reported widespread damage from adelgid infestations, but a national assessment is lacking. To date, little has been documented about adelgids in Bhutan. Schmutzenhofer (1985) may have been the first to do so, with a record of *Adelges* (*Dreyfusia*) on *Abies* at Thumsingla (now Phrumsengla) and *Pineus* on *Pin. wallichiana* near Bumthang in western Bhutan. Recently, Havill et al. (2025) described 4 new species of adelgids that were discovered as part of the survey discussed here.

Knowledge of worldwide adelgid diversity and biology is critical to prepare for early detection and rapid response if additional species become introduced outside of their native ranges. Indeed, some adelgid species have become devastating forest pests after being introduced out of their home range due to lack of co-evolved plant defenses and/or insufficient natural enemies. Some notable invasive adelgid pests are *Adelges* (*Annandina*) *tsugae* Annand, 1924 in North America on species of *Tsuga* (Havill et al. 2016a), *Adelges* (*Dreyfusia*) *piceae* (Ratzeburg 1844) in North America on species of *Abies* (Havill et al. 2021), and *Pineus* (*Pineus*) *pini* (Goeze 1778) and *Pineus* (*Pineus*) *boerneri* Annand, 1928 in many parts of the world on species of *Pinus*, especially where they have been used for plantation forestry (eg Rodas et al. 2015).

Besides their potential as pests, adelgids in Bhutan could also be sources of natural enemies for biological control programs elsewhere. Examples in the region include predators collected from adelgids on *Abies* and *Pinus* in India and Pakistan that were released as potential biological control agents of *Ad.* (*D.*) *piceae* in North Carolina, USA, but none became established (Amman and Speers 1971). Also, lady beetles (Coccinellidae) collected from *Ad.* (*An.*) *tsugae* on species of *Tsuga* in Sichuan and Yunnan Provinces, China (Yu et al. 2000) were introduced to the eastern United States but failed to become established or were not released because the lab colony was lost (Mayfield et al. 2023). Failure of predators from the region to establish was attributed to a poor match between the collecting and release sites (Amman and Speer 1971), or release of too few individuals (Montgomery and Havill 2014, Mayfield et al. 2023). The natural enemies feeding on adelgids in Bhutan could provide additional options for evaluation as biological control agents; especially dipterans, which were not a major focus of these past efforts.

Our survey for adelgids in Bhutan was prompted by the discovery of numerous light green strobile-shaped or pineapple galls that appeared to be retarding growth of *Pic. spinulosa* saplings in a forest nursery in Changaphu in 2019. In this paper, we summarize a country-wide survey of adelgids on potential conifer hosts. We discuss the diversity and distribution of adelgids, as well as observations about their biology. We also provide a summary of the dipteran natural enemies that were opportunistically found associated with the adelgid samples to provide some baseline information about their potential as biological control agents.

Materials and Methods

Recognizing the potential for economic impact of adelgids in conifer forests of Bhutan, the Asia Forest Cooperation Organization and National Institute of Forest Science, Republic of Korea supported a research project titled "Assessment of adelgid diversity and distribution in conifer forest of Bhutan to mitigate future outbreaks". Adelgids were collected between 2020 and 2022. Samples were collected in all months of the year except August and September, but most of the sampling effort occurred in the spring (February to May) and autumn (October to December) (Supplementary Table S1) in an attempt to document different parts of the adelgids' life cycles. Training of field surveyors was carried out for identification of adelgid infestation, collection, and transportation of samples. An online mobile data-gathering platform was created using the mobile application Epicollect5 (Aanensen et al. 2014) for surveyors to enter site coordinates and other biological information to maintain consistency in data collection. Samples were collected from sites where conifers are the dominant forest type, specifically in the districts of Bumthang, Haa, Mongar, Paro, Thimphu, Trashigang, Trashi Yangtse, and Wangdue Phodrang (Fig. 1). All but a few areas that were surveyed were natural areas and preserves (Supplementary Table S1). Potential hosts in the conifer genera used by adelgids were targeted, and whenever adelgids were observed, samples were collected.

Adelgids were left on, or separated from host plant material, stored in 99% ethanol, and refrigerated until they were shipped to the USDA Forest Service laboratory in Hamden, CT, USA for morphological examination and DNA barcoding. Immatures of predaceous flies (Diptera) were encountered with the ethanol-preserved adelgid materials, so these were also examined and DNA barcoded. In order to preserve morphological characters, DNA was nondestructively extracted from adelgid and fly specimens by piercing them with a sterilized insect pin and retaining the cuticles after proteinase K digestion. Extraction was performed using the ABI MagMAX DNA Multi-Sample Ultra Kit (Thermo Fisher Scientific) on a KingFisher instrument (Thermo Fisher Scientific). Sequences from the standard 658 bp DNA barcode section of the cytochrome c oxidase subunit I gene (COI) were generated using standard protocols (Foottit et al. 2009). Specimens cleared during DNA extraction, plus additional specimens from the same collections cleared with 10% KOH, were slide-mounted in Canada balsam. After examining the dipterans on slides and DNA barcodes, some were found to contain parasitic hymenoptera, which were also summarized.

Neighbor-joining trees were generated separately for adelgid DNA barcodes and for predator and parasitoid DNA barcodes. Sequences were aligned with Geneious Prime version 2023.2.1 (https://www.geneious.com), and neighbor-joining trees were constructed using uncorrected *p*-distance with 1,000 bootstrap replicated in PAUP* version 4.0a169 (Swofford 2003).

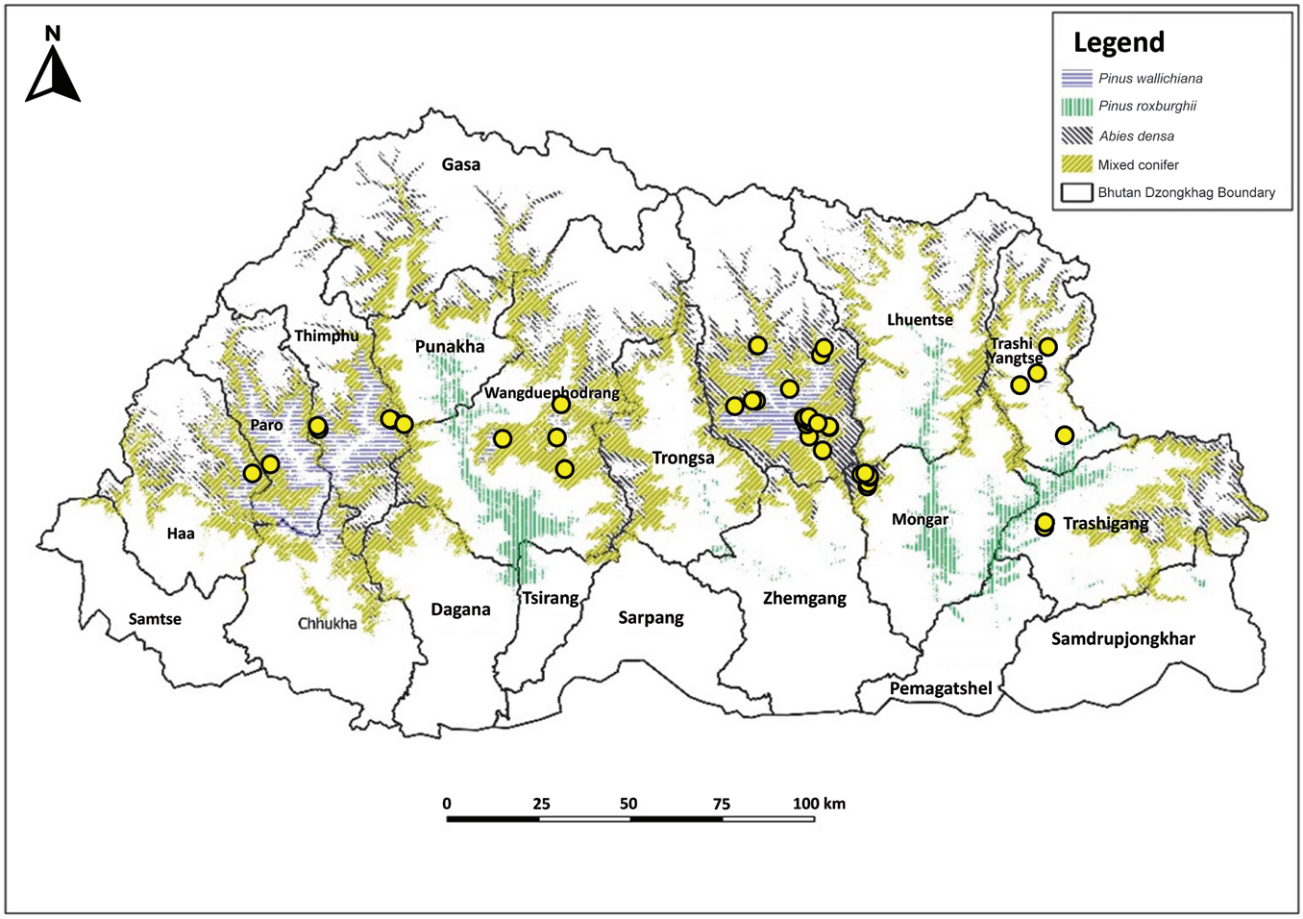

Fig. 1. Conifer forests of Bhutan and survey sampling sites where adelgids were observed and collected.

For predator and parasitoid DNA barcodes, clusters of specimens that differed by <2% sequence divergence were assigned as separate operational taxonomic units (OTUs). Sequences were searched on the Barcode of Life Database (BOLD: https://www.boldsystems.org) in June 2024 for their closest Barcode Index Number (BIN) and putative taxon membership. For cecidomyiid OTUs, genera were assigned by Dr. Raymond Gagné (USDA, retired) by comparing morphology of slide-mounted specimens to descriptions and known specimens (Gagné 1994). For chamaemyiid OTUs, genera were assigned based on their membership in clades that include morphologically identified adult species in a phylogeny of Chamaemyiidae that will be described in a subsequent paper (N. Havill and Stephen Gaimari, unpublished).

Adelgid colonies settled on *Pin. bhutanica* and *Pin. wallichiana* needles were often associated with prominent epiphytic fungi that appeared to be feeding on the adelgid honeydew (described in more detail in the Results and Discussion). To provide information about their identities, DNA sequences were generated from the associated fungi from two different sites (sample 22-155 from Tangsbi, Bumthang and sample 22-088 from Yangtse Range, Trashi Yangtse; Supplementary Table S1). Fungal hyphae were removed from pine needles on which adelgids were settled (Supplementary Table S1, samples 22-088 and 22-155) and fungal DNA was extracted using a modified glassmilk protocol (Jusino et al. 2014). PCR amplification and sequencing of the internal transcribed spacer (ITS) was performed with primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al 1990), and of the large subunit (28S) with primers LROR and LR6 (Vilgalys and Hester 1990), using the conditions in Karlsen-Ayala et al. (2023). Since ITS is used as a universal barcode region for fungal identification (Schoch et al. 2012), BLAST searches were conducted in GenBank for resulting sequences for initial identification. A maximum likelihood tree was reconstructed using 28S with RaxML v8.2.10 (Stamatakis 2014) using a GTR + G evolutionary model and branch support estimated with 1,000 bootstrap replicates. The tree included related sequences from Bose et al. (2014) and Chomnunti et al. (2014) and outgroup representatives of Mycosphaerellaceae that were downloaded from GenBank with accession numbers indicated in Supplementary Fig. S1.

Adelgid specimens from which DNA was nondestructively extracted, plus additional samples cleared with 10% KOH solution, were slide-mounted in Canada balsam. Adelgid and predator specimens were deposited at the National Biodiversity Centre (NBC), Ministry of Agriculture and Livestock, Thimphu, Bhutan, the Yale University Peabody Museum of Natural History (YPM), New Haven, CT, USA, the Canadian National Collection of Insects, Arachnids and Nematodes (CNC), Ottawa, ON, Canada, and the United States National Museum of Natural History (USNM), Beltsville, MD, USA. The locations of voucher specimens and their accession numbers (is assigned) are shown in Supplementary Tables S1 and S2. Photographs for the figures were generated with a DP28 digital camera mounted on an Olympus BX53 microscope. Image stacking was performed using Olympus CellSens v. 3.2 software, and *x*–*y* stitching was performed with Adobe Photoshop v. 23.3.2. Adelgid species were determined by comparing specimens to

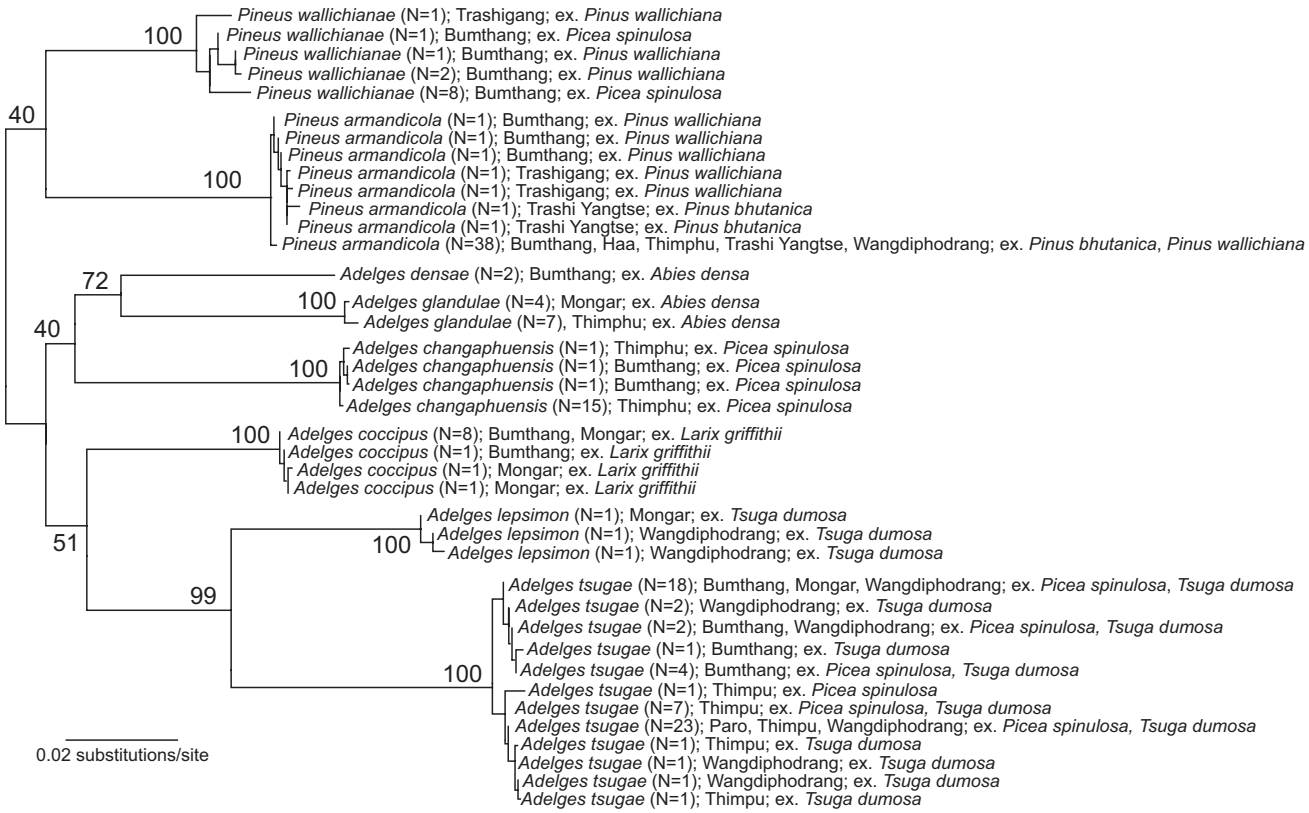

Fig. 2. Neighbor-joining tree of DNA barcode sequences from adelgids collected in Bhutan. For each tip, the species name is followed by number of specimens with that haplotype, source district, and host plant. Bootstrap support values are shown for each major cluster. Full collection information is in Supplementary Table S1.

published keys (Annand 1928, Favret and Aphid Taxon Community 2025), original descriptions (Annand 1924, Yaseen and Ghani 1971, Zhang et al. 1980, 1992, Havill et al. 2025), and high resolution photographs of type specimens of *Ad.* (*An.*) *tsugae*, and *Pineus* (*Pineus*) *wallichianae* Yaseen and Ghani, 1971 provided by USNM, and of *Adelges* (*Gillettella*) *glandulae* (Zhang, 1980) provided by the Institute of Zoology, Chinese Academy of Science (IZCAS).

Results and Discussion

Adelgid Diversity and Distribution in Bhutan

Adelgids were commonly present wherever host trees were observed during our survey. Samples from 43 sites were collected, examined morphologically, and analyzed using DNA barcode sequences (Fig. 1, Supplementary Table S1). The sequences were submitted to GenBank with accession numbers PQ013984 to PQ014145. We report 8 adelgid species in Bhutan, as supported by morphological examination and DNA barcoding. The neighbor-joining tree of DNA barcodes (Fig. 2) resulted in 8 species clusters with divergence among them ranging from 7.3% to 13.3%.

Four of the species were described elsewhere before our survey: *Ad.* (*An.*) *tsugae*, *Ad.* (*G.*) *glandulae*, *Pineus* (*Pineus*) *armandicola* Zhang, Zhong and Zhang, 1992, and *P.* (*P.*) *wallichianae*. The remaining 4 species were new to this survey and were described in a separate paper (Havill et al. 2025). These are: *Ad.* (*Cholodkovskya*) *changaphuensis* Havill and Brunet, 2025, *Ad.* (*Cassiadelges*) *coccipus* Havill and Brunet, 2025, *Ad.* (*Dreyfusia*) *densae* Havill and Brunet, 2025, and *Ad.* (*Annandina*) *lepsimon* Havill and Brunet, 2025. *Cassiadelges* Havill and Brunet, 2025 was also described as a new subgenus.

Adelgids were found on 6 of the 8 potential host species in Bhutan. Although adelgids were found in all the coniferous forests that we sampled in Bhutan, apparent damage was only observed in the spruce nursery at Changaphu in western Bhutan.

Two of the previously described adelgid species were known from east of Bhutan, in India and/or Pakistan [*Ad.* (*An.*) *tsugae*, *P.* (*P.*) *wallichianae*], and 3 were known from west of Bhutan, in southeastern China [*Ad.* (*An.*) *tsugae*, *Ad.* (*G.*) *glandulae*, and *P.* (*P.*) *armandicola*]. *Adelges* (*An.*) *tsugae* and *P.* (*P.*) *armandicola* were the most widely distributed species in our survey (Figs. 4I and 9F). For 2 species, our collections included morphological forms that have not been observed previously. These were adult exules of *Ad.* (*G.*) *glandulae* and adult fundatrices of *P.* (*P.*) *wallichianae*. Narratives with details about each adelgid species that was found in our survey follow. Information about the hosts plants and phylogenetic placement of the 4 new species in Bhutan is included with their species descriptions in Havill et al. (2025). We expand upon that here with additional information about their biology.

Adelges (Annandina) lepsimon Havill and Brunet, 2025

Adelges (*An.*) *lepsimon* was discovered feeding on *T. dumosa* at 3 sites in Mongar and 1 in Wangdue Phodrang (Fig. 3D, Supplementary Table S1). This species is sister to *Ad.* (*An.*) *tsugae*, the only other member of subgenus *Annandina* (Havill et al. 2025). *Adelges* (*Ad.*) *lepsimon* has a similar feeding habit and appearance to *Ad.* (*An.*) *tsugae*, settled at the base of the needles on current year's growth. The 2 species were collected near each other in Mongar and Wangdue Phodrang on *T. dumosa* but on different trees. Morphologically, the 2

species have similar adult exules (Figs. 3C and 4C), but first instars of *Ad.* (*An.*) *lepsimon* can be distinguished from *Ad.* (*An.*) *tsugae* by the presence of a distinct pair of wax glands on the head (Fig. 3A, Havill et al. 2025). *Adelges* (*An.*) *lepsimon* was less common than *Ad.* (*An.*) *tsugae*, being found only at 3 sites, while *Ad.* (*An.*) *tsugae* was found at 18 sites (Fig. 4I). In addition to exules of *Ad.* (*An.*) *lepsimon*, we also collected sexupara nymphs with wing pads (Fig. 3B), so it is likely that this species migrates to *Picea* where it completes a holocycle. Its sister species, *Ad.* (*An.*) *tsugae* completes its holocycle on *Pic. spinulosa* in Bhutan and *Pic. likiangensis* in China, so these might be species to survey for galls induced by *Ad.* (*An.*) *lepsimon.*

Adelges (Annandina) tsugae Annand, 1924

Adelges (*An.*) *tsugae* was the most widely distributed species in our survey. It was collected from *T. dumosa* in Bumthang, Mongar, Paro, Thimphu, and Wangdue Phodrang, and from *Pic. spinulosa* in Bumthang and Thimphu (Fig. 4I, Supplementary Table S1). On *T. dumosa*, it was found feeding on the bark of twigs, mostly at the base of needles, as is typical for the species (Havill et al. 2016b). Winged sexuparae were collected in late April in Uruk, Bumthang and Pelela, Wangdue Phodrang. On some branch tips, the adelgids reached very high densities on the stem (Fig.4H) but did not appear to affect tree health.

Picea spinulosa is a new primary host record for this species. The gall on *Pic. spinulosa* (Fig. 4G) is uniformly spherical (Fig. 4G), matching the morphology of *Ad.* (*An.*) *tsugae* galls on other *Picea* species in China, Taiwan, and Japan. For a single gall collected on 22 May 2022 in Thimpu (Supplementary Table S1, sample 22-177), we dissected out 910 gallicola nymphs, which are likely the offspring of a single fundatrix. The presence of sexuparae on *Tsuga* and matching DNA sequences from specimens collected on *Tsuga* and *Picea* constitute strong evidence for a holocycle for this species in Bhutan.

On *Pic. spinulosa*, we collected adult fundatrices (Fig. 4E) with eggs (Fig 4F) in early April, and fully formed galls (Fig. 4G) containing late instar nymphs in late May. The adult fundatrices are dark brown and they have red to dark purple eggs (Fig. 4F). Galls collected on 22 May 2022 ranged in size from 1.7 to 2.4 cm in diameter (n = 17, mean = 2.0 cm). In late October, the adelgids were present as fundatrix nymphs (third to fourth instar), the stage in which they appear to overwinter.

Adelges (*An.*) *tsugae* consists of a group of regionally divergent genetic lineages on different *Tsuga* and *Picea* host species in Japan, mainland China, Taiwan, Ulleung island, Korea, and western North America that should probably be split into multiple species or subspecies (Havill et al. 2016a). The samples from Bhutan represent an additional, previously unrecognized genetic lineage of *Ad.* (*An.*) *tsugae* that is sister to samples collected in western China (Havill et al. 2025). The DNA barcode haplotypes differ from the western Chinese haplotype by between 2.74% and 4.05 % uncorrected sequence divergence, suggesting that the lineage in Bhutan might be designated as a separate species or subspecies.

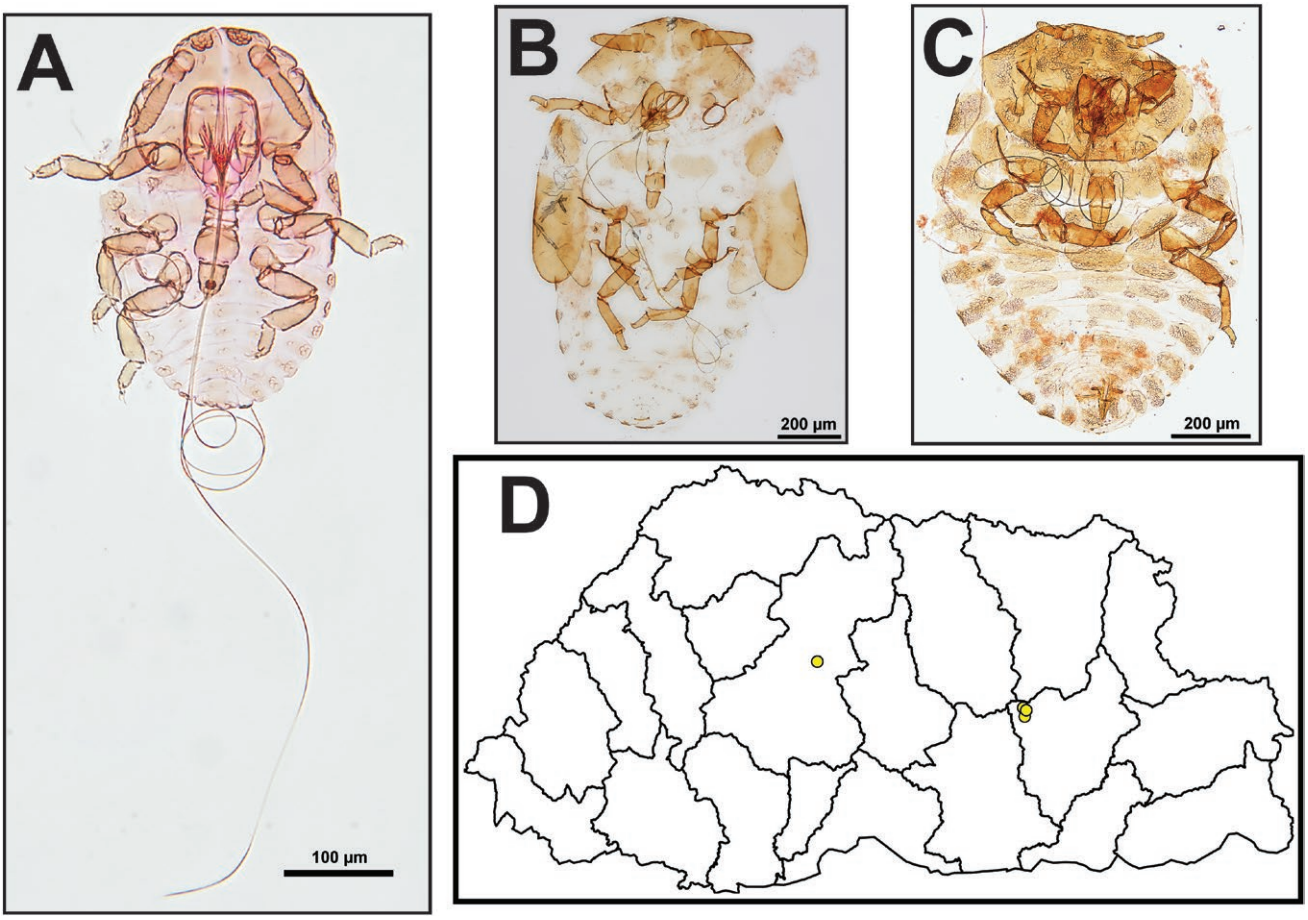

Fig. 3. *Adelges* (*Annandina*) *lepsimon.* A) Exulis nymphal first instar. B) Sexupara nymph. C) Exulis adult. D) Map of Bhutan showing collection sites. A to C modified from Havill et al. (2025).

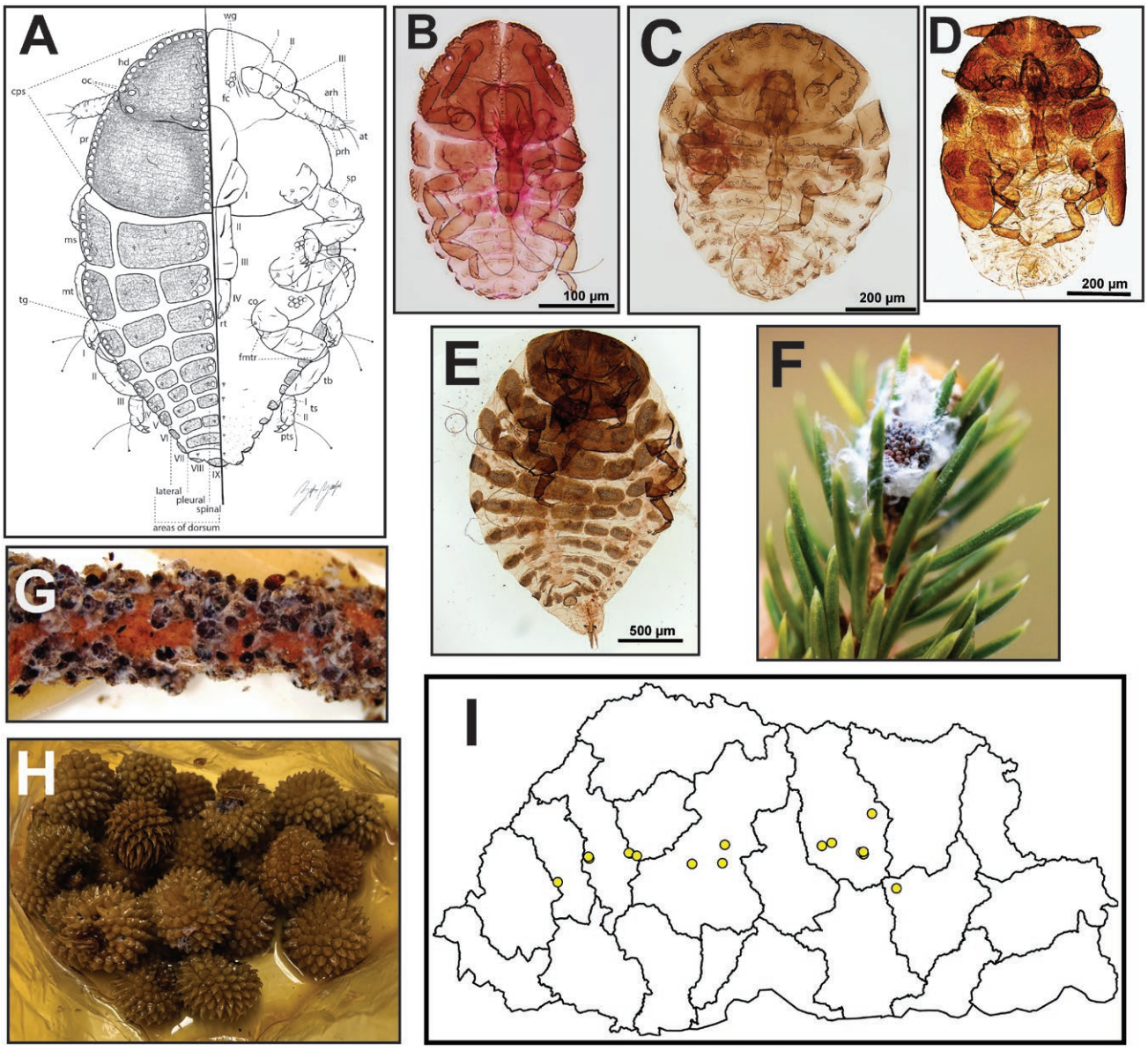

Fig. 4. *Adelges* (*Annandina*) *tsugae*. A) Illustration of exulis nymphal first instar. B) Exulis nymphal first instar. C) Exulis adult. D) Sexupara nymph. E) Fundatrix adult. F) Gallicola eggs on *Picea spinulosa*. G) Exules settled on *Tsuga dumosa* twig. H) Galls. I) Map of Bhutan showing collection sites.

Adelges (Dreyfusia) densae Havill and Brunet, 2025

Adelges (*D.*) *densae* was found at just one site in Bumthang feeding on *Ab. densa* (Fig. 5D, Supplementary Table S1). It feeds on the bark of the main stem and on the underside of twigs. The nymphal first instars have distinctive compound wax glands (Fig. 5A), and the later instar nymphs and adults have distinctive strongly sclerotized protuberant dorsal plates (Fig. 5B and C). We did not observe sexuparae in our collections, so there it is not known whether this species can complete a holocycle on *Picea*.

The *Dreyfusia* sp. specimens reported by Schmutzenhofer (1985) in central Bhutan were observed causing a downward twisting of *Ab. densa* needles. He considered these specimens the same as what Beeson (1941) called *Chermes abietis-piceae* Stebbing, 1903 in India. This species is now considered *nomen dubium* (Favret 2025), because its descriptions did not provide enough information to distinguish it from other species (Schneider-Orelli and Schneider 1954). It is possible that *Ad.* (*D.*) *densae*, *Ad.* (*G.*) *glandulae*, or a different species not detected in this survey could be what Schmutzenhofer (1985) reported, but we did not observe twisting of needles in our survey.

Adelges (Cassiadelges) coccipus Havill and Brunet, 2025

Adelges (*Ca.*) *coccipus* was collected from *L. griffithii* at 5 sites in Bumthang and Mongar (Fig. 6E, Supplementary Table S1). It settles on the base of buds, the base of needle whorls, and on the bark of twigs. This remarkable species is the first example in all of Aphidoidea that is completely legless (Havill et al. 2025). Similar to many scale insects, it has a first instar crawler stage with legs (Fig. 6A and B), and then loses them during the molt to the second nymphal instar (Fig. 6C). The vestigial legs consist of slight cuticular protrusions, each with a cluster of setae. Other appendages like the antennae and ovipositor are also reduced compared to most other adelgid species, suggesting pleiotropy in their developmental pathways. It has morphologically distinct sistens (Fig. 6A) and progrediens (Fig. 6B) forms in the first instar (Havill et al. 2025). The sistens specimens were damaged and collected earlier in the

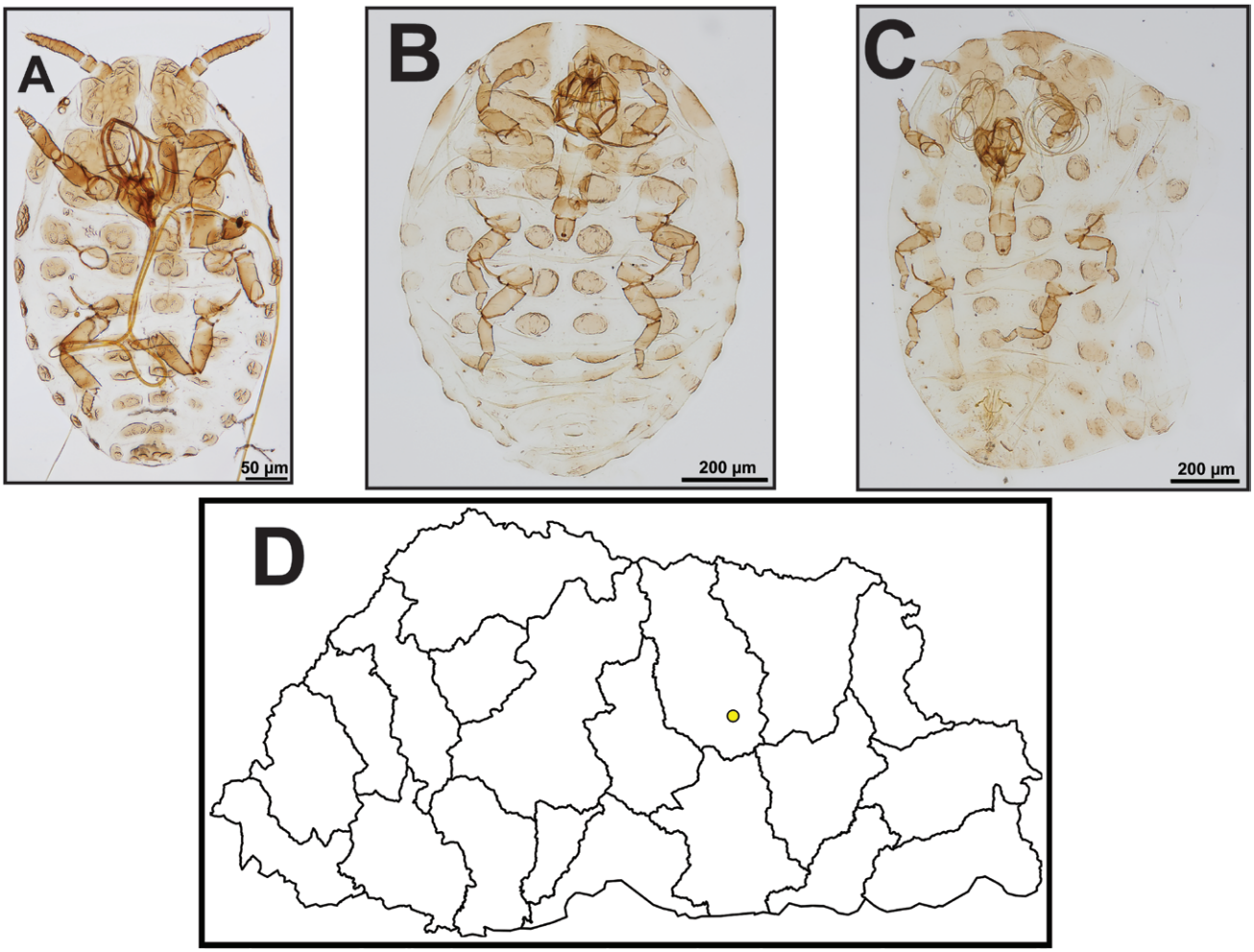

Fig. 5. *Adelges* (*Dreyfusia*) *densae*. A) Exulis nymphal first instar. B) Later exulis nymphal instar. C) Exulis adult. D) Map of Bhutan showing collection sites. A to C modified from Havill et al. (2025).

spring than progrediens forms, suggesting that they were the remnants of the overwintering generation that did not survive past the first instar. The first instar progrediens specimens were collected alive and later in the spring. It is not known whether these different forms also exhibit differences in development or behavior. In many other adelgid species, the sistentes have a diapause and all develop into wingless adults, while progredientes lack a diapause and can develop into either wingless individuals or winged sexuparae that migrate to *Picea* to complete a holocycle (Havill and Foottit 2007). We did not observe winged sexuparae, nor nymphs with wind pads, in *Ad.* (*Ca.*) *coccipus*, so it is unknown whether it can complete a holocycle. Additional observations throughout the year would be needed to determine its life cycle.

Adelges (Cholodkovskya) changaphuensis Havill and Brunet, 2025

Tree damage caused by *Ad.* (*Ch.*) *changaphuensis* at the Changaphu Forest Nursery (Fig. 7) prompted our wider survey of adelgids in Bhutan. The young *Pic. spinulosa* trees in the nursery suffered heavy infestation, which significantly retarded their growth, giving them a bushy look (Fig. 7A). Initially dismissed as young spruce cones, the galls received little attention, but the galls gradually matured and dried out, releasing the mature winged insects, giving the whole plant a dry brownish and ragged appearance (Fig. 7B). *Adelges* (*Ch.*) *changaphuensis* was collected from *Pic. spinulosa* at 1 site in Bumthang and 2 in Thimphu (Fig. 8G, Supplementary Table S1). It forms galls that have a strobile or pineapple shape (Fig. 8F) that is typical of many adelgids. The fundatrices overwinter as nymphs at the base of buds. In the spring, they develop to the adult stage and begin inducing the bud to develop into a gall. The adult fundatrices and their eggs are light green (Fig. 8C), in contrast to the dark brown fundatrix and red to dark purple eggs of *Ad.* (*An.*) *tsugae* (Fig. 4F) on the same host species. Similarly, the winged gallicolae that emerge from galls are light green (Fig. 8E), while those of *Ad.* (*An.*) *tsugae* are dark reddish brown. The life cycle of *Ad.* (*Ch.*) *changaphuensis* is not known. It could complete an anholocyclic life cycle only on *Picea*, or, since it is closely related to other adelgid species that use *Larix* species as secondary hosts (Havill et al. 2025), it could have a holocycle alternating between *Picea* and *Larix*. Observing whether the gallicolae can settle and reproduce on *Picea* and/or *Larix* could determine this.

Adelges (Gilletteela) glandulae (Zhang, 1980)

Adelges (*G.*) *glandulae* was first described by Zhang et al. (1980) in Sichuan, China from exules on *Abies fabri* (Mast.) Craib, and galls and their associated generations on *Pic. brachytyla* and *Pic. likiangensis*. Despite describing generations on both *Abies* and *Picea*, they concluded that this species was anholocyclic because exulis nymphs on *Abies* did not continue to develop after overwintering. In Bhutan, we collected *Ad.* (*G.*) *glandulae* from *Ab. densa* in Mongar

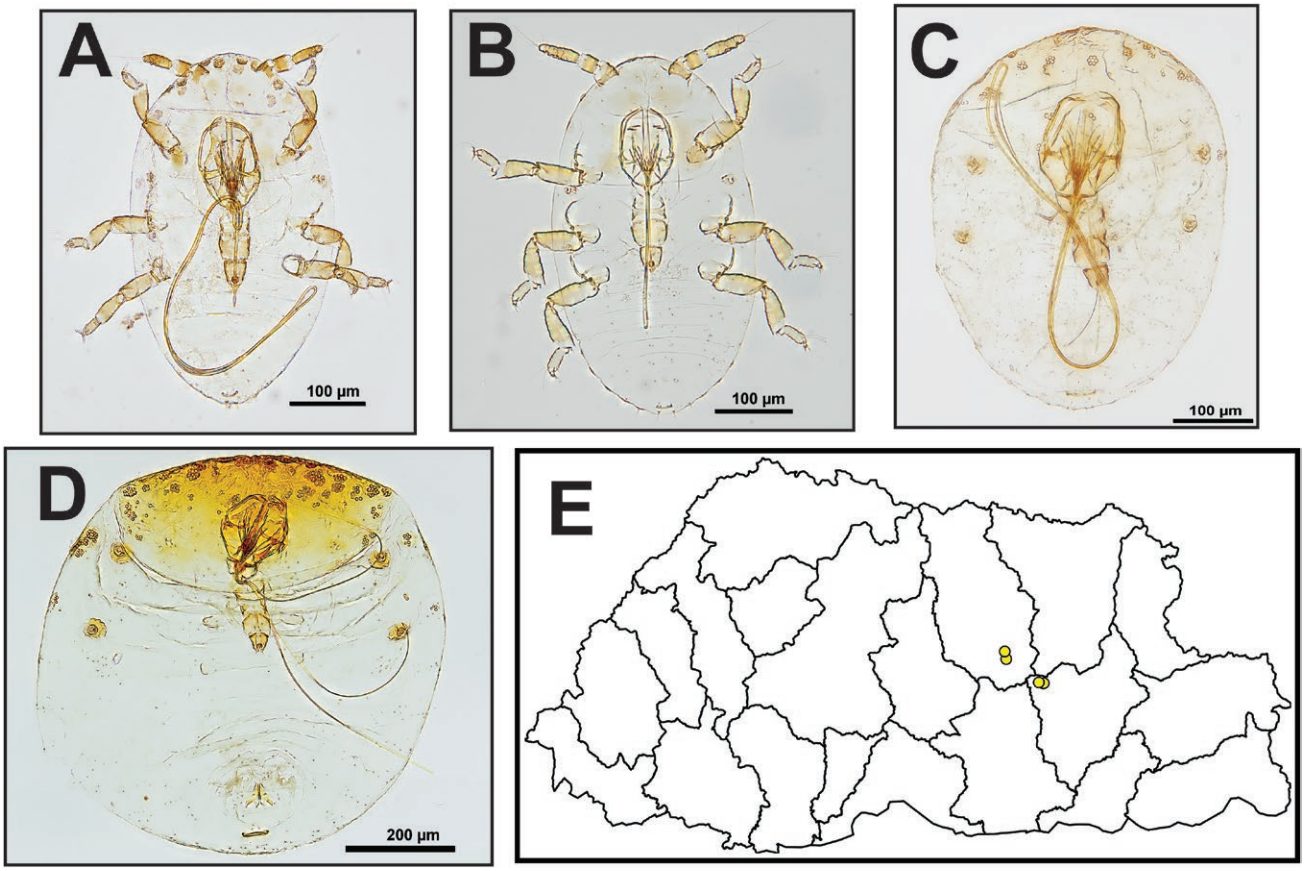

Fig. 6. *Adelges* (*Cassiadelges*) *coccipus*. A) Sistens nymphal first instar. B) Progrediens nymphal first instar. C) Exulis nymphal second instar. D) Exulis adult. E) Map of Bhutan showing collection sites. A to D modified from Havill et al. (2025).

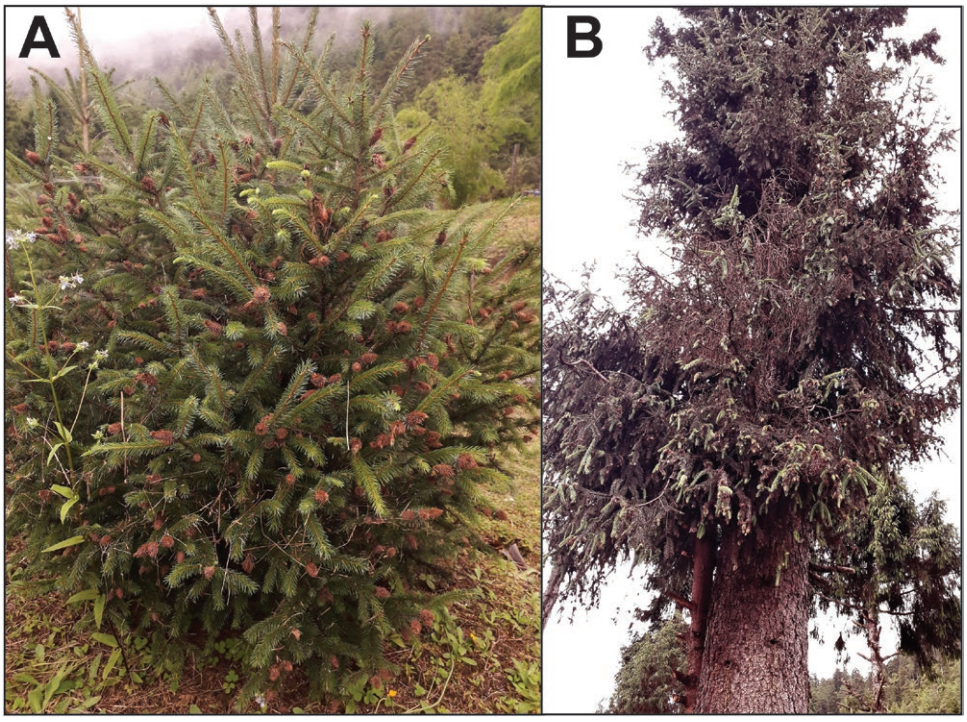

Fig. 7. *Picea spinulosa* infested with *Ad.* (*Ch.*) *changaphuensis* at a forest nursery in Changaphu. A) Sapling (approx. 1.5 m tall) with many fresh galls, showing reduced growth, and bushy appearance. B) Mature tree showing brownish and ragged appearance after repeated infestation.

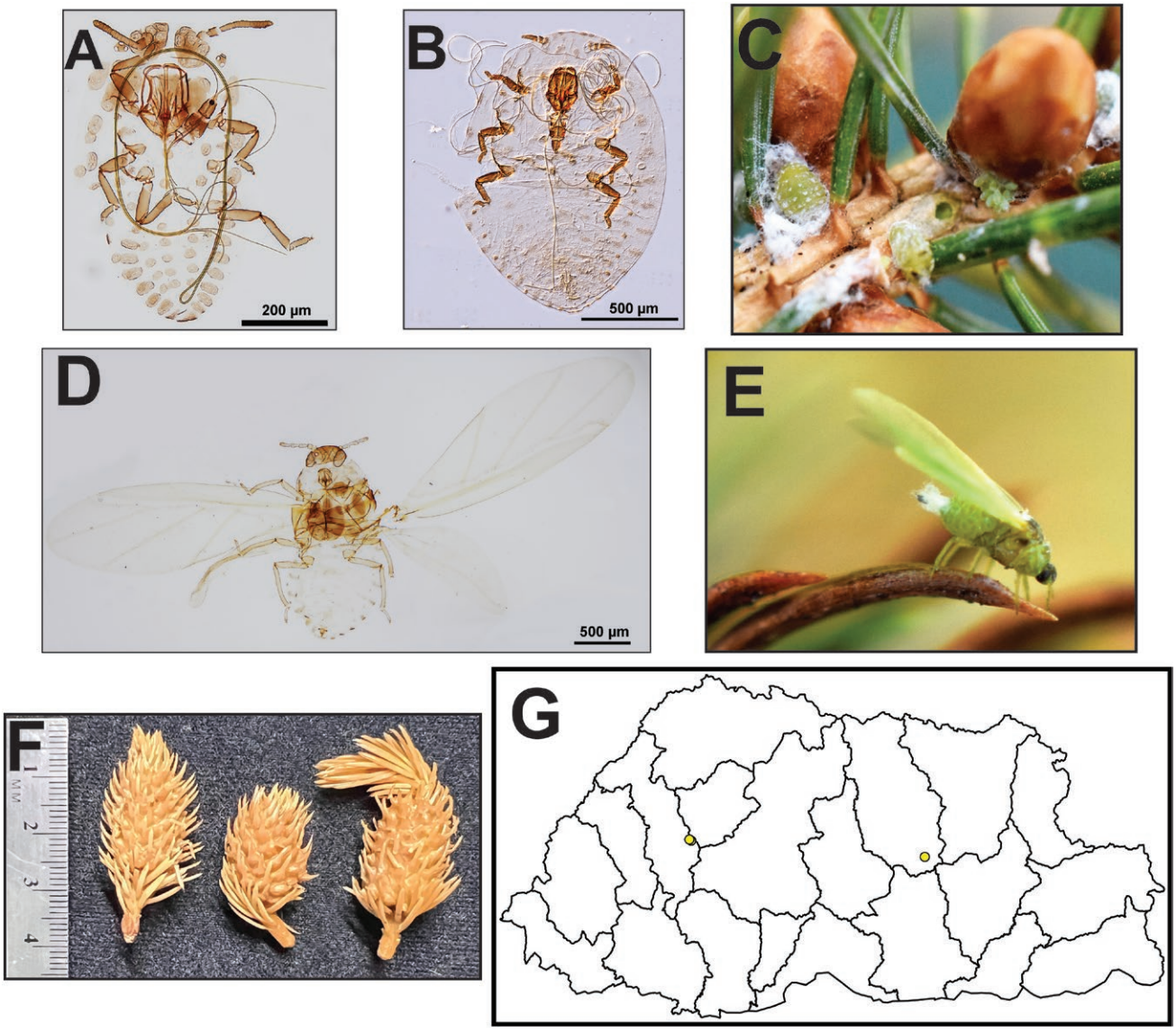

Fig. 8. *Adelges* (*Cholodkovskya*) *changaphuensis*. A) Fundatrix nymphal first instar. B) Fundatrix adult. C) Fundatrices with eggs settled on *Picea spinulosa*. D) Gallicola adult. E) Gallicola adult on *Picea spinulosa*. F) Galls. G) Map of Bhutan showing collection sites. A, B, D, and F modified from Havill et al. (2025).

and Thimphu (Fig. 9E, Supplementary Table S1), settled in dense colonies on the bark of small branches and at the base of needles on twigs (Fig. 9D). The DNA barcode sequences from our study diverged from sequences published on GenBank identified as *Ad.* (*G.*) *glandulae* collected in China by 0% (GenBank Accession EF073069) and 2.8% (GenBank Accession EF073072), indicating that there could be undiscovered biological variation in this species. In addition to nymphs (Fig. 9A and B), our samples included exulis adults from 2 sites (Fig. 9C), which were previously unknown, indicating that unlike what was reported in China, the overwintering exules can develop to the adult stage in Bhutan. We did not find specimens matching this species on *Picea*, so additional collecting effort on potential primary hosts are needed to determine the life cycle in Bhutan.

Pineus (Pineus) armandicola Zhang, Zhong and Zhang, 1992

Pineus (*P.*) *armandicola* is a holocyclic species that alternates between *Pin. armandii* Franch and *Pic. likiangensis* in Shaanxi, Sichuan, and Yunnan, China (Zhang et al. 1992, Li et al. 2002, Havill et al. 2007, Foottit et al. 2009, Chen and Qiao 2012). In Yunnan, *P.* (*P.*) *armandicola* is considered as an important defoliator of *Pin. armandii*. With advanced levels of infestation, the pine trees get covered by sooty mold that grows on the honeydew produced by the adelgids, causing tree damage and mortality (Li et al. 2002, Luo et al. 2002).

Despite *P.* (*P.*) *armandicola* being encountered across a wide geographic area in our survey, defoliation was not observed. It was collected from *Pin. wallichiana* at 16 sites in Bumthang, Haa, Thimpu, Trashigang, and Wangdue Phodrang, and from *Pin. bhutanica* at 5 sites in Trashi Yangtse (Fig. 10F, Supplementary Table S1). Our specimens from Bhutan are the furthest east that this species has been recorded, and *Pin. bhutanica* and *Pin. wallichiana* are new host records. Although closely related, the 2 allopatric pine species grow in different regions of the country. We did not collect *P.* (*P.*) *armandicola* from *Picea*, suggesting it could be anholocyclic on pines in Bhutan.

During our survey, this species was collected from the main trunk, base of the fascicles of needles, and on the needles of young pine trees.

At advanced infestation levels, the five needles of the pine fascicle were adhered together into a tube by honeydew and a sheath formed by fungal hyphae (Fig. 10D and E), and the adelgids were settled inside the tube. The pine needles also had a blackish appearance due to growth of sooty mold on the honeydew produced by the adelgids. Small colonies with mostly immature insects (Fig 10A and B) did not have the fungus associated with them, suggesting that heavier adelgid densities are necessary to produce enough honeydew to initiate fungal growth. The fungal ITS and 28S sequences were submitted to GenBank under accession numbers PQ456142-PQ456143, and PQ456144-PQ456145, respectively. Their top BLAST results were accessions within the sooty mold family Capnodiaceae. Phylogenetic analysis of 28S (Supplementary Fig. S1) showed that the sample from Tangsbi, Bumthang (sample 22-155; Supplementary Table S1) could be a species of *Fumiglobus*, and the other sample from Yangtse Range, Trashi Yangtse (sample 22-088; Supplementary Table S1) could be a species of *Scorias*. Both of these genera lack robust molecular representation which makes species identification challenging and so it is unknown whether our samples represent described species. The 2 samples from which we obtained sequences were collected 58 km apart and were phylogenetically diverged, suggesting that the association between adelgids and fungi may not be species-specific. Rather, the fungi and adelgids might have a facultative relationship, with the adelgid honeydew providing nourishment and the fungus creating an environment protected from adverse weather and predators.

The unidentified *Pineus* species reported by Schmutzenhofer (1985) is likely *P.* (*P.*) *armandicola* because it was also described as being on *Pin. wallichiana* settled on needles that were "glued together" and covered with honeydew and that were "blackish-dark and sticky".

Pineus (Pineus) wallichianae Yaseen and Ghani, 1971

Pineus (*P.*) *wallichianae* is known to settle on the bark of the shady side of stems and undersides of twigs of mature and overmature *Pin. wallichiana* in West Pakistan (Yaseen and Ghani 1971). Its life cycle was reported as being anholocyclic on this host with 2 generations per year.

Our samples from Bhutan were collected from *Pin. wallichiana* at 2 sites in Bumthang and one in Trashigang, and from *Pic. spinulosa* at 2 sites in Bumthang (Fig. 11D, Supplementary Table S1). *Picea spinulosa* is a new host for this species and provides evidence of a possible holocycle, which was not previously known. Our exulis samples from *Pinus* are morphologically consistent with the description of

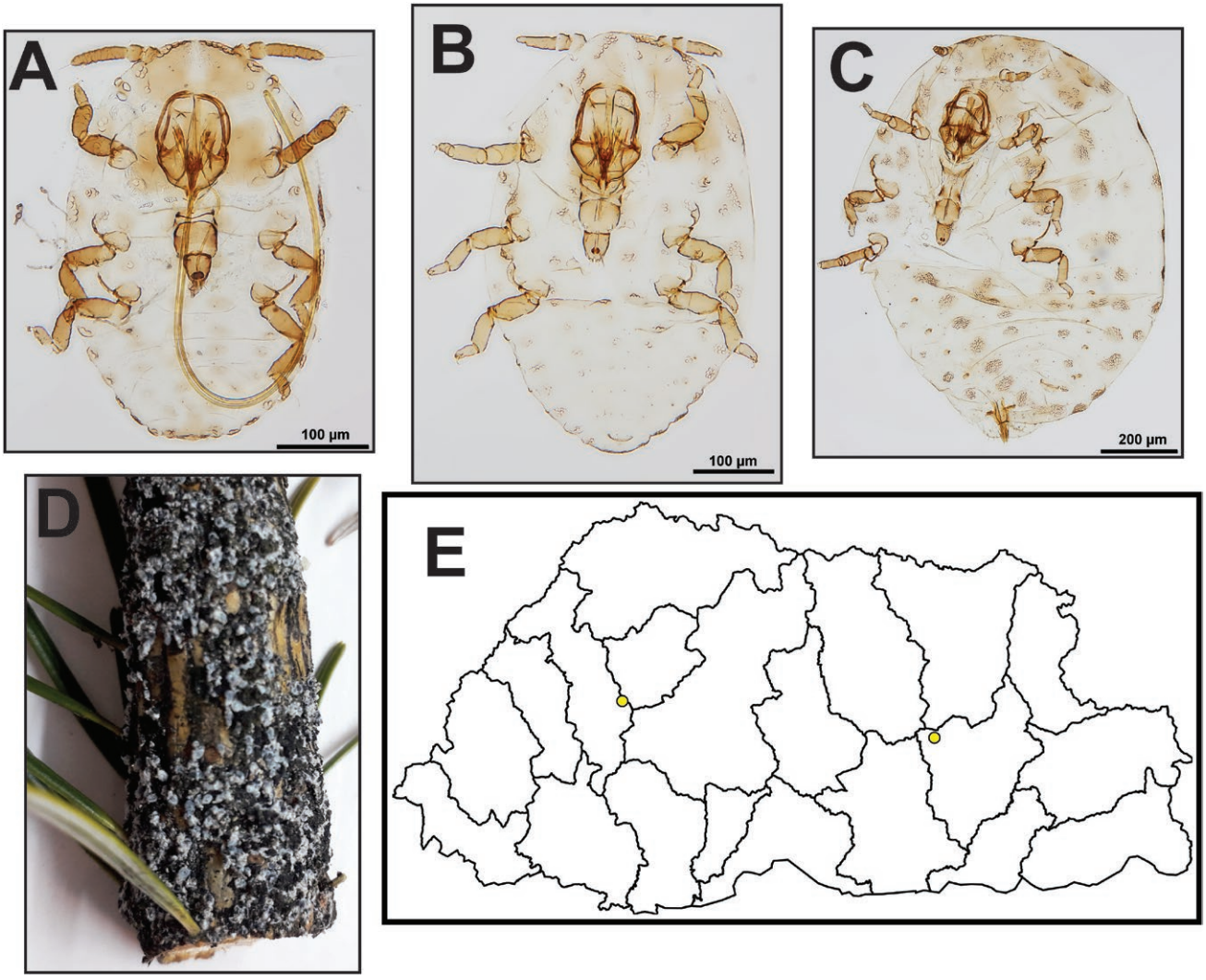

Fig. 9. *Adelges* (*Gilletteella*) *glandulae*. A) Exulis nymphal first instar. B) Later exulis nymphal instar. C) Exulis adult. D) Exules settled on bark of *Abies densa*. E) Map of Bhutan showing collection sites.

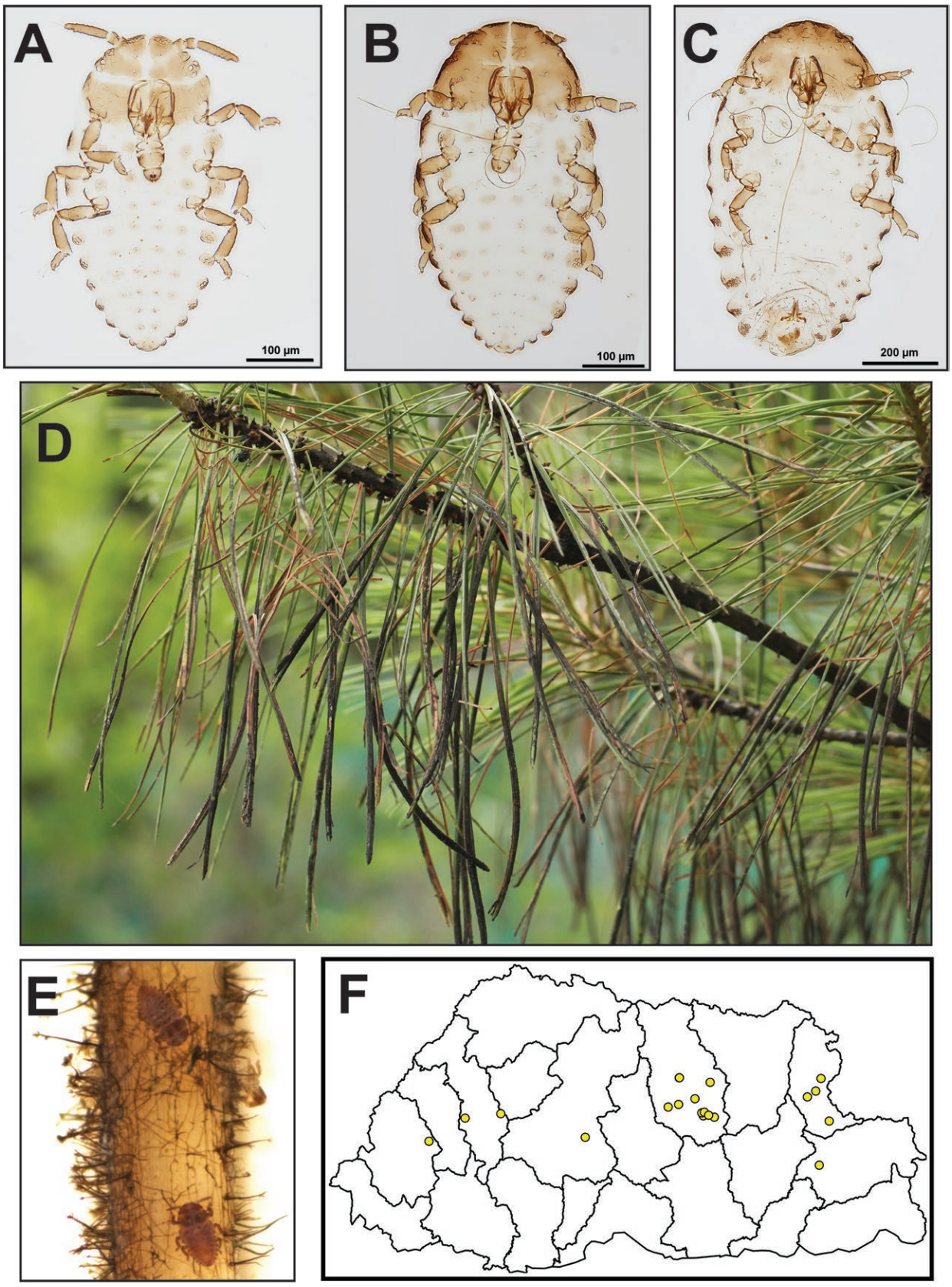

Fig. 10. *Pineus* (*Pineus*) *armandicola.* A) Exulis nymphal first instar. B) Later exulis nymphal instar. C) Exulis adult. D) Adelgid-infested *Pinus bhutanica* with fungus. E) Map of Bhutan showing collection sites.

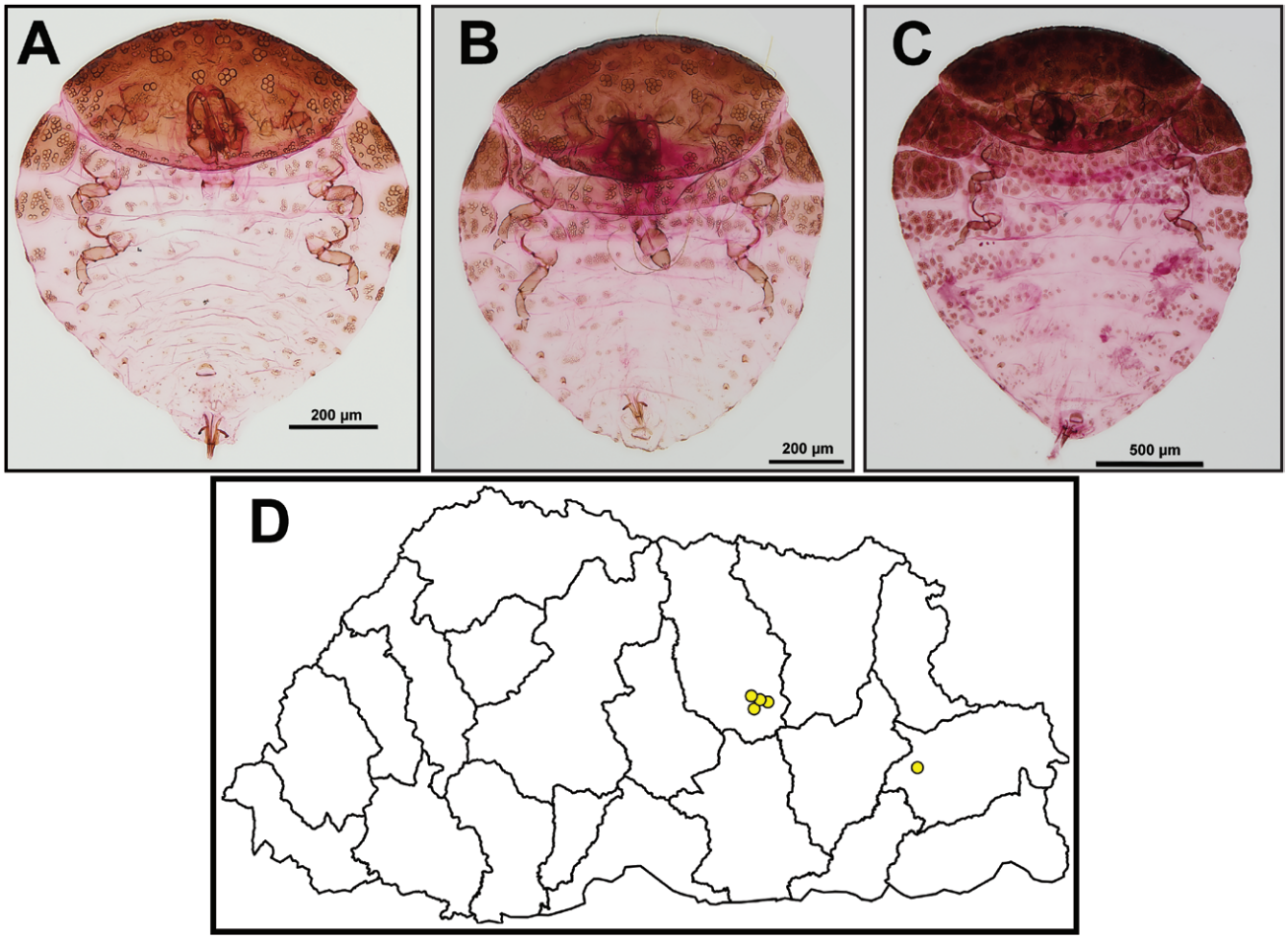

Fig. 11. *Pineus* (*Pineus*) *wallichianae*. A) Exulis adult from *Pinus wallichiana*. B) Exulis intermediate form adult from *Picea spinulosa*. C) Fundatrix adult from *Picea spinulosa*. D) Map of Bhutan showing collection sites.

Yaseen and Ghani (1971). Our samples from *Picea* were of 2 different forms. Specimens that were likely to be fundatrices were settled at the base of new buds. These buds were slightly swollen compared to buds lacking adelgids, so they may have begun to induce galls, but they were collected too early in the season (early April) for us to confirm whether this was the case. The adult fundatrices had very dense clusters of wax pores on the cephaloprothoracic shield (Fig. 11C) compared to the adult exules on *Pinus* (Fig. 10A). An additional form on *Picea* was found settled on the bark and under previous seasons' node scales of twigs (Fig. 11B), farther from buds than the putative fundatrices, so are not likely to be involved in gall formation. These adult specimens had an intermediate density of wax pores between the fundatrices (Fig. 11C) and the exules on *Pinus* (Fig. 11A). Additional sampling will be needed to observe the developmental and behavioral differences among the forms to fully describe life cycle in Bhutan. One possibility is that the intermediate forms settled on the bark could be a recent transition to an anholocyclic life cycle having only exules living on *Picea* without gall formation. Three other *Pineus* species have this type of life cycle, *Pineus* (*Pineus*) *konowashiyai* Inouye, 1945, *Pineus* (*Pineus*) *patchiae* Börner, 1926, and *Pineus* (*Pineus*) *pineoides* (Cholodkovsky, 1903).

Adelgid Predators

Immatures predaceous flies associated with adelgids in our survey were in 3 families: Cecidomyiidae, Chamaemyiidae, and Syrphidae. These are the same 3 families commonly encountered in other surveys of adelgid natural enemies (eg Wilson 1938, Pschorn-Walcher and Zwölfer 1956, Wantuch et al. 2019), including in the Himalayan region (Rao and Ghani 1972, Krishnaswamy and Chacko 1988).

We collected 19 cecidomyiid, 140 chamaemyiid, and 14 syrphid individuals within ovisacs or adjacent to settled adelgids (Table 1, Figs. 12 and 13, Supplementary Table S2). Of these, we successfully generated DNA barcodes for 7 cecidomyiids, 103 chamaemyiids, and 13 syrphids. The sequences were submitted to GenBank with accession numbers PQ045271 to PQ045393 (Supplementary Table S2). Two cecidomyiids and 2 chamaemyiids harbored endoparasitoids as indicated by DNA barcodes that matched hymenopteran parasitoid families. Three additional chamaemyiid larvae had parasitoid larvae that could be seen inside their slide-mounted specimens (eg Fig. 13B).

The neighbor-joining tree of DNA barcodes for adelgid predators and their parasitoids (Fig. 12) resulted in 14 clusters of sequences with <2% uncorrected sequence divergence, which we designated as separate OTUs. We did not recover any adult predators in this survey, and our DNA barcodes did not have close matches with unambiguously identified specimens in BOLD, so we were not able to identify any to species.

All but one of the cecidomyiid larvae were identified morphologically as *Lestodiplosis*. The one unknown larva was damaged, so could not be determined. This genus contains predators of insects and mites, and along with *Aphidoletes*, includes common adelgid predators (Gagné 1989). There were 3 cecidomyiid OTUs with DNA

Table 1. Summary of dipteran predators and hymenopteran parasitoids associated with each adelgid species in our survey. Predator family was determined morphologically. Parasitoids, indicated with an asterisk (*) are nested in the dipteran taxa from which they were recovered. Predator and parasitoid taxa within families were determined using DNA barcodes. "Unknown" indicates no barcode was generated, so only family could be determined. Detailed specimen information is in Supplementary Table S2

Adelgid species	Predator family	Predator or *parasitoid taxon	No. individuals
Adelges (*Annandina*) *lepsimon*	Cecidomyiidae	*Lestodiplosis*	2
	Syrphidae	Unknown	1
Adelges (*Annandina*) *tsugae*	Cecidomyiidae	*Lestodiplosis*	8
		Lestodiplosis sp. B	1
		Unknown	1
		*Platygastridae sp. A	2
	Chamaemyiidae	Unknown	9
		Leucopis sp. D	4
	Syrphidae	Unknown	1
		Parasyrphus sp. A	4
Adelges (*Cassiadelges*) *coccipus*	Cecidomyiidae	*Lestodiplosis* sp. C	3
	Chamaemyiidae	Unknown	3
		Neoleucopis sp. J	4
Adelges (*Cholodkovskya*) *changaphuensis*	Cecidomyiidae	*Lestodiplosis*	1
	Syrphidae	Unknown	1
		Melangyna sp. A	1
Adelges (*Dreyfusia*) *densae*	Syrphidae	*Melangyna* sp. A	6
Adelges (*Gilletteella*) *glandulae*	Syrphidae	Unknown	1
Pineus (*Pineus*) *armandicola*	Cecidomyiidae	*Lestodiplosis* sp. A	1
	Chamaemyiidae	Unknown	28
		Leucopis sp. D	5
		Neoleucopis sp. H	45
		Neoleucopis sp. I	40
		Neoleucopis sp. J	1
		*Figitidae sp. A	1
		*Pteromalidae sp. A	1
	Syrphidae	*Episyrphus* sp. A	2
Pineus (*Pineus*) *wallichianae*	Chamaemyiidae	*Neoleucopis* sp. K	3

barcodes. *Lestodiplosis* sp. A was a larva (Fig. 13F) found with *P.* (*P.*) *armandicola*. Its DNA barcode sequence had a 99% match to a BOLD BIN that included unidentified cecidomyiids collected in Primorskiy Kray, Russia, and in the Republic of Georgia, suggesting that this OTU has a rather wide Asian distribution. The 2 other cecidomyiids with DNA barcodes, *Lestodiplosis* sp. B, found with *Ad.* (*An.*) *tsugae*, and *Lestodiplosis* sp. C, found with *Ad.* (*Ca.*) *coccipus*, had closest matches in BOLD of 6% and 4%, respectively, so no additional biological information is available about them.

The abundance and diversity of Chamaemyiidae suggest that these predators could be especially important for regulating adelgid populations in Bhutan. There was one OTU in *Leucopis* and 4 in *Neoleucopis*, with their putative genus designations assigned according to their nested positions in a large unpublished phylogenetic data set for this group (N. Havill and Stephen Gaimari, unpublished). *Leucopis* D (Fig. 13A) included 4 larvae found with *Ad.* (*An.*) *tsugae*, and 5 larvae found with *P.* (*P.*) *armandicola*. *Neoleucopis* sp. H (Fig. 13B) included 10 puparia and 35 larvae, and *Neoleucopis* sp. I (Fig. 13C) included 39 larvae. Both of these species were found exclusively on *P.* (*P.*) *armandicola*. *Neoleucopis* sp. J (Fig. 13E) included 4 larvae from *Ad.* (*Ch.*) *coccipus*, and 1 larva from *P.* (*P.*) *armandicola*. *Neoleucopis* sp. K (Fig. 13E) included 3 larvae from *P.* (*P.*) *wallichianae*.

The syrphid OTUs were tentatively placed in genera based on close matches to BOLD BINS with identified individuals. The 2 larval specimens of *Episyrphus* sp. A (Fig. 13H) collected from *P.* (*P.*) *armandicola* had a 99% match to a BOLD BIN that contains specimens previously collected broadly in the Palearctic region, including in Bhutan, some of which were identified as *Episyrphus balteatus* (De Geer 1776). This is a common species throughout the Palearctic region that feeds on aphids and their relatives (Speight 2017). It has been recorded feeding on adelgid species, *Ad.* (*D.*) *nordmannianae* Eckstein 1890 in Poland (Ravn et al. 2012). The 4 larvae of *Parasyrphus* sp. A (Fig. 13J) were collected inside *Ad.* (*An.*) *tsugae* galls. Their closest match in BOLD was 3% to a BIN that includes specimens of *Parasyrphus punctulatus* (Verrall 1873), a widely distributed species throughout the Palearctic region (Speight 2017). Our specimens could be this or a closely related species. Feeding by dipteran predators inside tightly closed adelgid galls, like those of *Ad.* (*An.*) *tsugae* is uncommon but has been reported (Mitchell and Maksymov 1977). The 6 specimens (6 eggs and 3 larvae) of *Melangyna* sp. A (Fig. 13I) were collected from *Ad.* (*D.*) *densae*, and 1 larva was collected from *Ad.* (*Ch.*) *changaphuensis*. These fell into 2 clusters in the neighbor-joining tree that differed by 2% sequence divergence. We placed them into the same OTU, but they may represent 2 species. Their closest match in BOLD was 3% to a BIN that included *Melangyna pavlovskyi* Violovitsh 1956, which is another widely distributed Palearctic species (Speight 2017).

The 2 cecidomyiid larvae that returned parasitoid DNA barcodes were collected from *Ad.* (*An.*) *tsugae*. The closest match in BOLD was 5% to a BIN with specimens identified as Playtgastridae. The 2 chamaemyiid puparia with parasitoid DNA barcodes were collected from *P.* (*P.*) *armandicola*. One had a DNA barcode that was 9% diverged from a BIN with specimens of Figitidae and the other was 4% diverged from a BIN with specimens of Pteromalidae. Chamaemyiid puparia, for the most part, cannot be identified morphologically to genus or species, but the puparium parasitized by a putative pteromalid could be *Neoleucopis* sp. H, because other

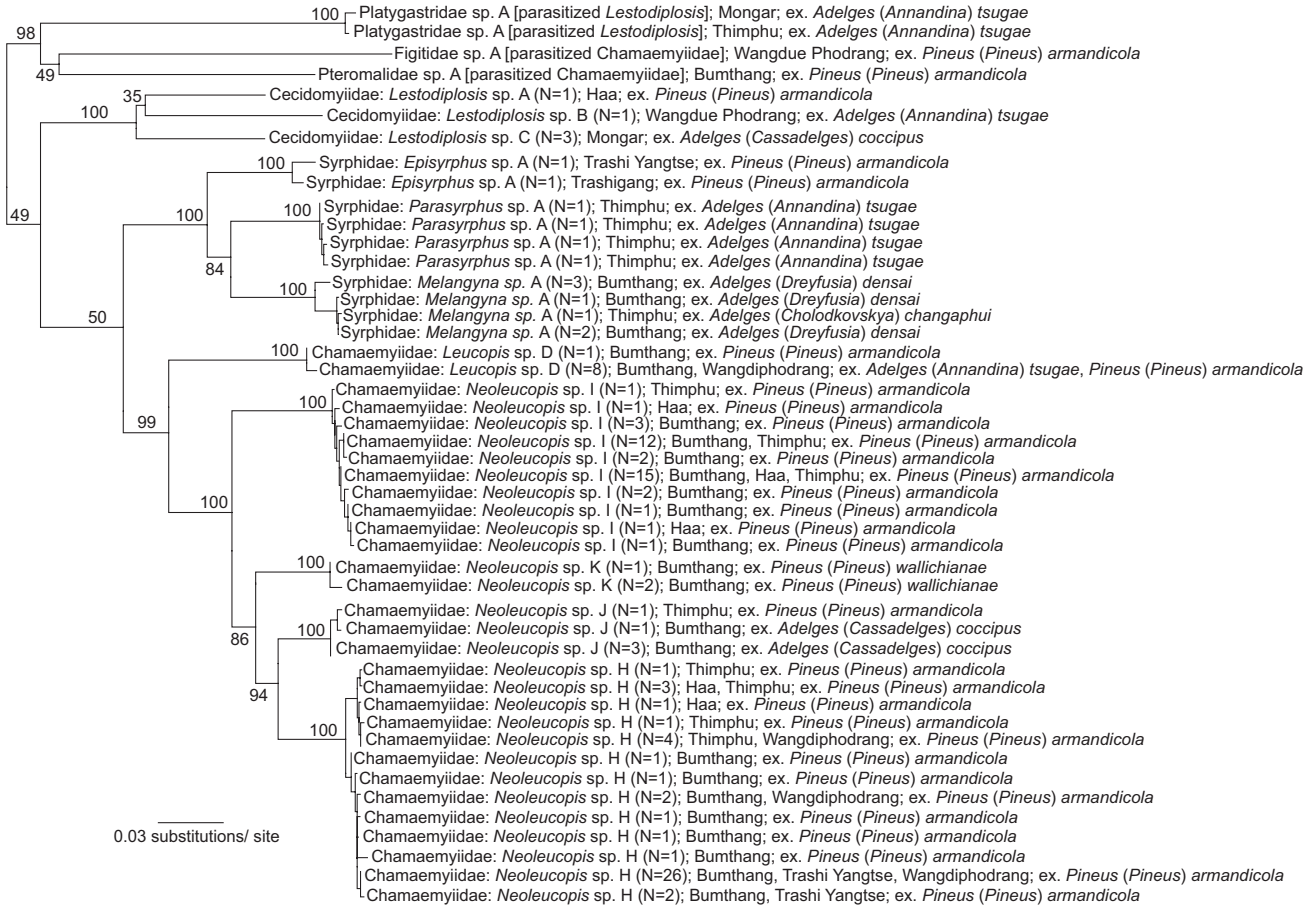

Fig. 12. Neighbor-joining tree of DNA barcode sequences from predators and their parasitoids associated with adelgids collected in Bhutan. For each tip, the taxon name is followed by number of specimens with that haplotype, source district, and adelgid prey species. Full collection information is in Supplementary Table S2. Bootstrap support values are shown for each major cluster.

specimens from the same sample were identified as this OTU. All 3 chamaemyiid larvae with parasitoids evident inside their slide-mounted specimens had DNA barcodes that identified them as *Neoleucopis* sp. H collected from *P.* (*P.*) *armandicola*.

Figitidae and Pteromalidae are the 2 parasitoid families that were also recovered from 2 *Leucotaraxis* (Chamaemyiidae) species native to western North America being used for biological control of *Ad.* (*An.*) *tsugae* in eastern North America (Kohler et al. 2008, Celis et al. 2022). The recovery of these same 2 families in distant regions may indicate that these families include the most common parasitoids of chamaemyiids.

These findings about adelgid predators in Bhutan can generate new research interest in using predacious flies for biological control of adelgids. For example, the diversity and abundance of chamaemyiids found associated with *P.* (*P.*) *armandicola*, might provide options for biological control of this species in China, where it is a significant pest. In addition, *Ad.* (*An.*) *tsugae* from Japan is a high-impact pest of *Tsuga* in eastern North America, where there is an effort to evaluate and import biological control organisms from the adelgid's native ranges (Havill et al. 2016b, Mayfield et al. 2023). Despite not being the origin of the introduction to North America, *Ad.* (*An.*) *tsugae* in Bhutan could be another potential source of biological control agents because it has similar biology to the invasive Japanese lineage of *Ad.* (*An.*) *tsugae*. More work would have to be competed to determine the host ranges of predators from this region and assess whether they can successfully prey on *Ad.* (*An.*) *tsugae* on North American hemlocks.

There are undoubtedly taxa of adelgid predators other than flies in Bhutan, but our sampling was biased toward those that are less likely to be dislodged during collection. Other predator groups within Coleoptera, Hemiptera, and Neuroptera are also commonly associated with adelgids (eg Rao and Ghani 1972). Therefore, there is a need for further exploration to target adult predators, including adult flies, that can be identified and matched to the DNA barcodes that we generated from immatures. This will provide a more comprehensive inventory of the diversity of insects that feed on adelgids to understand their population dynamics and assess their potential use for biological control.

Conclusions

In Bhutan, heavy damage at the stand level was observed at Changaphu in Thimphu, whereas, from rest of the country, damage at the stand level was not apparent. However, proper damage assessment is required to accurately measure the extent of impact of adelgids on the conifer forest in Bhutan. Our survey results provide valuable information about the adelgid fauna and their predators as a springboard for additional work on the impacts of adelgids on forest health in Bhutan and the suitability of their predators as biological control agents.

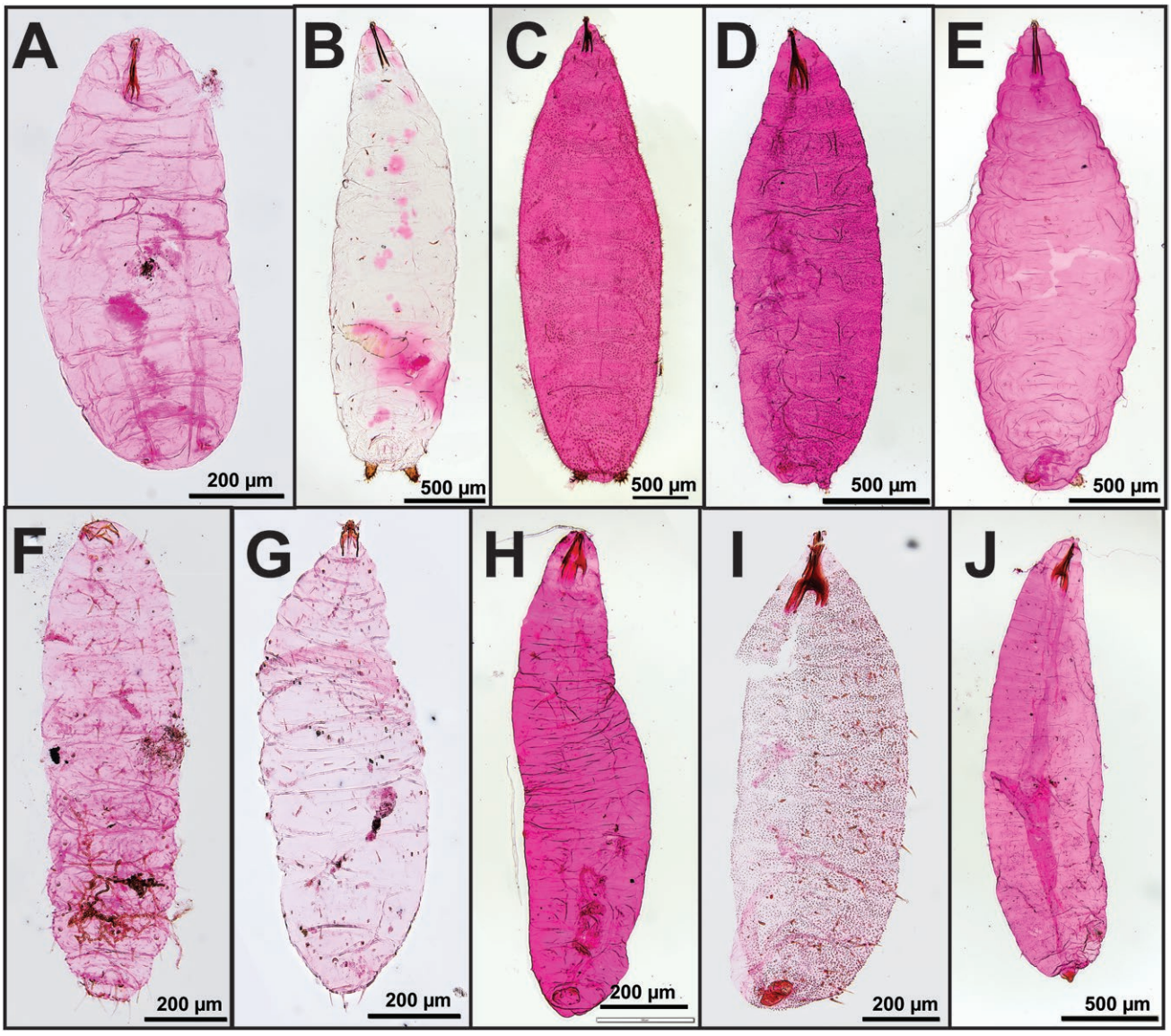

Fig. 13. Examples of predaceous dipteran larvae recovered in our survey. Sample number is in brackets and complete collection information is in Supplementary Table S1. A) *Leucopis* sp. D [21-048-05]. B) *Neoleucopis* sp. H with hymenopteran endoparasitoid larva [22-117-01]. C) *Neoleucopis* sp. I [22-144-03]. D) *Neoleucopis* sp. J [22-165-03]. E) *Neoleucopis* sp. K [22-163-01]. F) *Lestodiplosis* sp. A [22-113-01]. G) *Lestodiplosis* [22-115-01]. H) *Episyrphus* sp. A [22-129-01] . I) *Melangyna* sp. A [22-159-04]. J) *Parasyrphus* sp. A [22-179-02].

Supplementary material

Supplementary material is available at *Journal of Insect Science* online.

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Authors contribution

Kaka Tshering (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Supervision [equal], Writing—original draft [lead], Writing—review & editing [equal]),

Namgay Shacha (Investigation [supporting], Writing—review & editing [supporting]), Tshewang Norbu (Investigation [supporting], Writing—review & editing [supporting]), Yonten Norbu (Investigation [supporting], Writing—review & editing [supporting]), Tshering Tenzin (Investigation [supporting], Writing—review & editing [supporting]), Sonam Tashi (Investigation-Supporting, Writing—review & editing [supporting]), Zephyr Zembrzuski (Data curation [equal], Investigation [supporting], Visualization [equal], Writing—review & editing [supporting]), Elena Karlsen-Ayala (Formal analysis [supporting], Investigation [supporting], Methodology [supporting], Writing—review & editing [supporting]), Nathan Havill (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Funding

acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Resources [equal], Supervision [equal], Visualization [equal], Writing - review & editing [lead])

Conflicts of interest. None declared.

References

Aanensen DM, Huntley DM, Menegazzo M, et al. 2014. EpiCollect+: linking smartphones to web applications for complex data collection projects. F1000Res 3:199. https://doi.org/10.12688/f1000research.4702.1

Amman GD, Speers CF. 1971. Introduction and evaluation of predators from India and Pakistan for control of the balsam woolly aphid (Homoptera: Adelgidae) in North Carolina. Can. Entomol. 103:528–533. https://doi.org/10.4039/ent103528-4

Annand PN. 1924. A new species of *Adelges* (Hemiptera, Phylloxeridae). Pan-Pac. Entomol 1:79–82.

Annand PN. 1928. A contribution toward a monograph of the Adelginae (Phylloxeridae) of North America. Stanford University Press (Stanford University Publications. University Series. Biological Sciences).

Beeson CFC. 1941. Ecology and control of the forest insects of India and the neighbouring countries. Vasant Press.

Börner C. 1926. Züchtung der Homopteren. Handbuch der biologischen Arbeitsmethoden 9:15–270.

Bose T, Reynolds DR, Berbee ML. 2014. Common, unsightly and until now undescribed: *Fumiglobus pieridicola* sp. nov., a sooty mold infesting *Pieris japonica* from western North America. Mycologia 106:746–756. https://doi.org/10.3852/13-288

Celis SL, Dietschler NJ, Bittner TD, et al. 2022. Hymenopteran Parasitoids of *Leucotaraxis argenticollis* (Diptera: Chamaemyiidae) and *Leucotaraxis piniperda*: implications for biological control of hemlock woolly adelgid (Hemiptera: Adelgidae). Environ. Entomol. 51:901–909. https://doi.org/10.1093/ee/nvac060

Chen J, Qiao G-X. 2012. Galling aphids (Hemiptera: Aphidoidea) in China: diversity and host specificity. Psyche 2012:1–11. https://doi.org/10.1155/2012/621934

Cholodkovsky NA. 1903. Aphidologische Mittheilungen. Zool. Anz. 26:258–263.

Chomnunti P, Hongsanan S, Aguirre-Hudson B, et al. 2014. The sooty moulds. Fungal Divers. 66:1–36. https://doi.org/10.1007/s13225-014-0278-5

Chou M-H, Shen Z-Y, Chu I-H, et al. 2025. Species delimitation and biogeography of Adelgidae (Hemiptera): phylogeographic origin of newly discovered Adelges in Taiwan and their North American sister. Insect Syst Div 9. https://doi.org/10.1093/isd/ixaf008

De Geer C. 1776. Memoires pour servir a l'histoire des insects, Vol. 6. P. Hesselberg. 523p.

Eckstein K. 1890. Zur Biologie der Gattung *Chermes* L. Zool. Anz. 13:86–90.

Favret C, Aphid Taxon Community (eds.). 2025. Blackman & Eastop's Aphids on the World's Plants, version 1.0. Available from https://aphidsonworldsplants.info

Favret C. 2025. Aphid species file. Available from: http://Aphid.SpeciesFile.org

Favret C, Havill NP, Miller GL, et al. 2015. Catalog of the adelgids of the world (Hemiptera, Adelgidae). Zookeys 534:35–54. https://doi.org/10.3897/zookeys.534.6456

Foottit RG, Maw HEL, Havill NP, et al. 2009. DNA barcodes to identify species and explore diversity in the Adelgidae (Insecta: Hemiptera: Aphidoidea). Mol. Ecol. Resour. 9:188–195. https://doi.org/10.1111/j.1755-0998.2009.02644.x

Forest Monitoring and Information Division. 2023. National Forest Inventory, Volume I, State of Forest Report. Department of Forest and Park Services, Ministry of Energy and Natural Resources, Royal Government of Bhutan. 160 p.

Gagné RJ. 1989. The Plant-Feeding Gall Midges of North America. Cornell University Press. 356 p.

Gagné RJ. 1994. The gall midges of the neotropical region. Cornell University Press. 352 p.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113–118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x

Goeze, JAE. 1778. Entomologische Beyträge zu des Ritter Linné Zwölften Ausgabe des Natursystems, Vol. 2. Weidmann Erben und Reich. 352 pp.

Grierson AJC, Long DG. 1983. Flora of Bhutan, including a record of plants from Sikkim, Volume 1, Part 1. Royal Botanical Garden. 186 p.

Havill NP, Foottit RG. 2007. Biology and evolution of Adelgidae. Annu. Rev. Entomol. 52:325–349. https://doi.org/10.1146/annurev.ento.52.110405.091303

Havill NP, Foottit RG, von Dohlen CD. 2007. Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. Mol. Phylogenet. Evol. 44:357–370. https://doi.org/10.1016/j.ympev.2006.11.008

Havill NP, Shiyake S, Lamb Galloway A, et al. 2016a. Ancient and modern colonization of North America by hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae), an invasive insect from East Asia. Mol. Ecol. 25:2065–2080. https://doi.org/10.1111/mec.13589

Havill NP, Vieira LC, Salom SM. 2016b. Biology and control of hemlock woolly adelgid. USDA Forest Service, Forest Health Technology Enterprise Team.

Havill NP, Griffin BP, Andersen JC, et al. 2021. Species delimitation and invasion history of the balsam woolly adelgid, *Adelges (Dreyfusia) piceae* (Hemiptera: Aphidoidea: Adelgidae), species complex. Syst. Entomol. 46:186–204. https://doi.org/10.1111/syen.12456

Havill NP, Brunet B, Zembrzuski Z, et al. 2025. Four new adelgid (Hemiptera: Adelgidae) species from Bhutan, including the first legless Aphidomorpha. Zootaxa 5583:437–461. https://doi.org/10.11646/zootaxa.5583.3.2

Inouye M. 1945. Monographische Studie über die Japanischen Koniferen-Gallenläuse (Adelgidae). Bull. Hokkaido Forestry Exp. Station 15:1–105.

Jusino MA, Lindner DL, Cianchetti JK, et al. 2014. A minimally invasive method for sampling nest and roost cavities for fungi: a novel approach to identify the fungi associated with cavity-nesting birds. Acta Ornithol. 49:233–242. https://doi.org/10.3161/173484714x687127

Karlsen-Ayala E, Jusino MA, Gazis R, et al. 2023. Habitat matters: the role of spore bank fungi in early seedling establishment of Florida slash pines. Fungal Ecol. 62:101210. https://doi.org/10.1016/j.funeco.2022.101210

Kohler GR, Stiefel VL, Wallin KF, et al. 2008. Parasitoids reared from predators of hemlock woolly adelgid (Hemiptera: Adelgidae), and the hymenopterous parasitoid community on western hemlock in the Pacific Porthwest. Environ. Entomol. 37:1477–1487. https://doi.org/10.1603/0046-225x-37.6.1477

Krishnaswamy S, Chacko MJ. 1988. Population dynamics of *Adelges knucheli* and its predators in the northwestern Himalayas, I: host phase: gallicola. J. Biol. Control 2:92–96.

Li Y, Xie K, Cao K, et al. 2002. A study on the biology of the *Pineus armandicola* (Homoptera: Adelgidae). Scientia Silvae Sinicae 38:82–87.

Luo C, Chen Y, Xie K, et al. 2002. Study on relationship between occurrence of *Pineus armandicola* and environmental factors. For. Res. 15:111–115.

Mayfield AE III, Bittner TD, Dietschler NJ, et al. 2023. Biological control of hemlock woolly adelgid in North America: history, status, and outlook. Biol. Control 185:105308. https://doi.org/10.1016/j.biocontrol.2023.105308

Mitchell RG, Maksymov JK. 1977. Observations of predation on spruce gall aphids within the gall. Entomophaga 22:179–186. https://doi.org/10.1007/bf02377841

Montgomery ME, Havill NP. 2014 Balsam woolly adelgid. In: Van Driesche R, Reardon R, editors. The use of classical biological control to preserve forests in North America. FHTET-2013-2. USDA Forest Service, Forest Health Technology Enterprise Team. p. 9–19.

Pschorn-Walcher H, Zwölfer H. 1956. The predator complex of white-fir woolly aphids (genus Dreyfusia, Adelgidae). Zeitschrift angevandt Entomologischtik 39:63–75.

Rao VP, Ghani MA (eds.) 1972. Studies on predators of *Adelges* spp. in the Himalayas; miscellaneous publication No. 3. Farnham Royal. Commonwealth Agricultural Bureaux.

Ratzeburg JTC. 1844. Die forst-insecten oder Abbildung und beschreibung der in den wäldern Preussens und der nachbarstaaten als schädlich oder nützlich bekannt gewordenen insecten; in systematischer folge und mit besonderer rücksicht auf die vertilgung der schädlichen. Nicolaische buchhandlung. 314 pp.

Ravn HP, Havill NP, Akbulut S, et al. 2012. *Dreyfusia nordmannianae* in Northern and Central Europe: potential for biological control and

comments on its taxonomy. J. Appl. Entomol. 137:401–417. https://doi.org/10.1111/jen.12012

Rodas CA, Serna R, Bolaños MD, et al. 2015. Biology, incidence and host susceptibility of *Pineus boerneri* (Hemiptera: Adelgidae) in Colombian pine plantations. South. For. 77:165–171. https://doi.org/10.2989/20702620.2014.1001662

Schmutzenhofer H. 1985. Technical Advisory Service for Forestry Development. Consultancy in Forest Entomology. FO:BHU/83/022. Food and Agriculture Organization.

Schneider-Orelli O, Schneider F. 1954. *Dreyfusia*-Befall an *Abies pindrow* im Nordwesthimalaya. Mitt. Schweiz. Entomol. Ges. 27:413–422.

Schoch CL, Seifert KA, Huhndorf S, et al; Fungal Barcoding Consortium. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. U.S.A. 109:6241–6246. https://doi.org/10.1073/pnas.1117018109

Speight MCD. Species accounts of European Syrphidae, 2017. Syrph the Net, the database of European Syrphidae (Diptera). Vol. 97. Syrph the Net publications. 294 p.

Stamatakis A. 2014. RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stebbing EP. 1903. A first note on the life-history of *Chermes abietis-piceae* Steb. M. S. J. Asiat. Soc. Bengal 72:229–235.

Swofford DL. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates.

Verrall GH. 1873. Additions and correction to the list of British Syrphidae. Entomol. Mon. Mag. 9:251–256. https://doi.org/10.5962/bhl.part.4732

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Violovitsh NA. 1956. Novye vidy roda *Syrphus Fabr*. (Diptera, Syrphidae) s Dalnego Vostoka. Zool. Zh. 35:741–745. [In Russian]

Wantuch HA, Havill NP, Hoebeke ER, et al. 2019. Predators associated with the pine bark adelgid (Hemiptera: Adelgidae), a native insect in Appalachian forests, United States of America, in its southern range. Can. Entomol. 151:73–84. https://doi.org/10.4039/tce.2018.53

White TJ, Bruns TD, Lee SB, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al., editors. PCR protocols: a guide to methods and applications. Academic Press. p. 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wilson F. 1938. Notes on the insect enemies of Chermes with particular reference to *Pineus pini*, Koch, and *P. strobi*, Hartig. Bull. Entomol. Res. 29:373–389. https://doi.org/10.1017/s0007485300026286

Yaseen M, Ghani MA. 1971. Descriptions, and notes on the biology, of four new species of Adelgidae from West Pakistan. Bull. Entomol. Res. 61:191–205. https://doi.org/10.1017/s0007485300057722

Yu G, Montgomery ME, Yao D. 2000. Lady beetles (Coleoptera: Coccinellidae) from Chinese hemlocks infested with the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae). Coleopterists Bull. 54:154–199. https://doi.org/10.1649/0010-065x(2000)054[0154:lbccfc]2.0.co;2

Zhang G-X, Zhong T-S, Tian Z-J. 1980. Two new species and a subspecies of Adelgidae from Sichuan, China. Zool. Res. 1:381–388.

Zhang G-X, Zhong T-S, Zhang W-Y. 1992. Homoptera: Aphidoidea. In: Chen S, editor. Insects of the Hengduan Mountains region, Volume 1. Science Press. p. 360–403.